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$mGluR_{1,5}$ activation protects cortical astrocytes and GABAergic neurons from ischemia-induced impairment

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ABSTRACT

Ischemic neuron death is presumably caused by excitotoxicity. Here, we studied whether ischemia impaired astrocytes and GABAergic neurons to exacerbate glutamate-dependent neural excitotoxicity by electrophysiologically recording these nerve cells in cortical slices. Our results showed that ischemia impaired the activity of glutamate-transporters (Glu-T) on the astrocytes, as well as the ability of firing spikes and the response to excitatory synaptic inputs on GABAergic neurons. The impairments of glutamate reuptakes and GABAergic neurons led to the imbalance between excitation and inhibition toward neural excitotoxicity. When explored the protection of nerve cells from ischemia, we found that the ischemic impairments of astrocytes and GABAergic cells were prevented by 3,5-DHPG, an agonist for type-I/V of metabotropic glutamate receptors (mGluR). The activation of $mGluR_{1,5}$ is likely a potential therapeutic strategy to prevent nervous tissues from excitotoxicity by reducing the impairment of the astrocytes and GABAergic neurons during the early stage of ischemia.

1. Introduction

Neuronal excitotoxicity in the early stage of ischemia is presumably reason for initiating brain cells' death (Choi, 1988; Won et al., 2002), in which the glutamate-dependent elevations of intracellular Ca^{2+} and free radicals are involved (Block, 1999; Lipton, 1999; White et al., 2000; Schwartz-Bloom and Sah, 2001; Welsh et al., 2002; Metha et al., 2007). The efforts to correct these deficits have not improved stroke patients (Taoufik and Probert, 2008; Candelario-Jalil, 2009). Other mechanisms and therapeutic strategies for ischemia need to be explored.

The impairments of GABAergic cells and glutamate reuptake led to inhibition weakness, extracellular glutamate accumulation and neuronal excitotoxicity (Johansen and Diemer, 1991; Saji et al., 1994; Centonze et al., 2001; Muller et al., 2001; Chen and Swanson, 2003; Kanai and Hediger, 2003; Wang, 2003; Dronne et al., 2007; Chen et al., 2010b; Huang et al., 2010). Ischemia elevated Ca^{2+} and proton in GABAergic neurons (Mitani et al., 1995; Simon and Xiong, 2006; Huang et al., 2010) and changed the expression of astrocytic glutamate transporters (Inage et al., 1998; Swanson et al., 2004; Camacho and Massieu, 2006; Yamashita et al., 2006). Thus, we examined how ischemia impaired the functions of astrocytes and GABAergic neurons for neuronal excitotoxicity.

In terms of the protection of the astrocytes and GABAergic neurons from the functional impairment, we focused on studying the role of metabotropic glutamate receptors (mGluR). The rationale for this study was based on the following studies. mGluR were localized at the cortical neurons and astrocytes (Wisniewski and Car, 2002; McNamara et al., 2006). The applications of mGluR modulators led to the diverse effects on the cultured neurons under the conditions of NMDA receptor activation and oxygen-glucose deprivation (Strasser et al., 1998; Pellegrini-Giampietro et al., 1999; Bruno et al., 2001; Werner et al., 2007). The type-I/V of mGluR is located on postsynaptic cells (Ferraguti et al., 2008; Zhang et al., 2012). In these regards, we examined whether $mGluR_{1,5}$ activation protected cortical astrocytes and GABAergic neurons from ischemic injury.

2. Materials and methods

2.1. Brain slices and neurons

Entire procedures were approved by Institutional Animal Care and Use Committee in Harbin Heilongjiang, China. Cortical slices (400 μM) were prepared from FVB-Tg(GadGFP)45704Swn/J mice

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(Jackson Lab, USA) that somatostatin-positive GABAergic cells were labeled genetically by green fluorescent protein (GFP). The mice in postnatal days 19–21 were anesthetized by inhaling isoflurane and decapitated by a guillotine. Cortical slices were sectioned with a Vibratome in the oxygenated (95% O_2 and 5% CO_2) artificial cerebrospinal fluid (ACSF) in the concentrations (mM) of 124 NaCl, 3 KCl, 1.2 NaH_2PO_4, 26 $NaHCO_3$, 0.5 $CaCl_2$, 4 $MgSO_4$, 10 dextrose, and 5 HEPES, pH 7.35 at 4 °C. These slices were held in the oxygenized ACSF (124 NaCl, 3 KCl, 1.2 NaH_2PO_4, 26 $NaHCO_3$, 2.4 $CaCl_2$, 1.3 $MgSO_4$, 10 dextrose and 5 HEPES, pH 7.35) at 25 °C for 1 h. A slice was transferred to a submersion chamber (Warner RC-26G) that was perfused with the oxygenated ACSF at 31 °C for whole-cell recording (Wang and Kelly, 2001). Chemical reagents were purchased from Sigma.

Cortical astrocytes were selected under DIC microscope (Nikon, FN-E600, Japan) based on their small somata and multiple fine processes in star-like shape. The astrocytes had low input resistance and resting membrane potentials around −90 mV, and did not produce action potentials. Cortical GABAergic neurons for whole-cell recording in the layer II–III of sensory cortex were selected to be GFP-labeled neurons under a DIC/fluorescent microscope (Nikon, FN-E600), in which an excitation wavelength was 488 nm. These neurons demonstrated fast spiking and less adaptation in spike amplitude and frequency, i.e., the typical properties of interneurons (Freund and Buzsaki, 1996; McKay and Turner, 2005; Wang et al., 2008, 2009; Ni et al., 2010; Yu et al., 2012).

2.2. Whole-cell recording and cellular functions

GABAergic neurons were recorded by an AxoPatch-200B amplifier under current-clamp for studying their intrinsic properties, and under voltage-clamp for analyzing their responses to excitatory synaptic inputs. Astrocytes were recorded under voltage-clamp for analyzing glutamate transporter (Glu-T) currents. Electrical signals were inputted into pClamp 10 (Axon Instrument Inc., CA, USA) for data acquisition and analyses. The output bandwidth in this amplifier was 3 kHz. Pipettes for whole-cell recordings were filled with the standard solution including (mM) 150 K-gluconate, 5 NaCl, 5 HEPES, 0.4 EGTA, 4 Mg-ATP, 0.5 Tris-GTP, and 5 phosphocreatine (pH 7.35 adjusted by 2 M KOH). Fresh pipette solution was filtered with centrifuge filters (0.1 μM). Osmolarity was 295–305 mOsmol, and pipette resistance was 5–6 MΩ (Chen et al., 2008).

The functions of GABAergic neurons were estimated in our study based on their intrinsic properties (Chen et al., 2006a, 2006b) and their responses to excitatory synaptic inputs (Wang, 2003). Sequential spikes at these GABAergic neurons were induced by injecting depolarization pulses (one second). Their ability of firing the spikes was estimated by measuring input–output, in which the correlations were plotted between stimulus intensities and spikes for these GABAergic neurons (Chen et al., 2006a, 2006b; Wang et al., 2008).

The responsiveness of GABAergic neurons to excitatory synaptic inputs were evaluated by recording their spontaneous excitatory postsynaptic currents (sEPSC) under whole-cell voltage-clamp without presynaptic stimulation (Wang and Kelly, 2001; Wang, 2003; Wang and Zhang, 2004). The cumulative probability of sEPSC amplitudes presents neuronal responsiveness to excitatory synaptic inputs. The recorded sEPSCs were blocked by 10 μM 6-Cyano-7-nitroquinoxaline-2,3-(1H,4H)-dione (CNQX, SIGMA) in certain experiments to make sure the currents to be glutamatergic.

The functions of astrocytes were evaluated through recording their Glu-T currents under whole-cell voltage-clamp. Glu-T currents on astrocytes were evoked by stimulating presynaptic axon (Bergles and Jahr, 1997), with which the astrocytes terminated on the postsynaptic neurons (Zoli and Agnati, 1996; Nagy et al., 2004). 10 μM DL-threo-β-Benzyloxyaspartate (an antagonist of Glu-T from TOCRIS; (Shimamoto and Lebrun, 1998; Bridges and Esslinger, 2005) was applied to the slices in certain experiments to make sure Glu-T currents.

2.3. In vitro ischemia

To simulate artery's occlusion and intracranial anastomotic circulation during in vivo ischemic stroke, we reduced the perfusion rate to cortical slices from 2 ml/min to 0.2 ml/min for 6 min in the experiments (Wang, 2003). We recorded action potentials and synaptic transmission on GABAergic neurons, as well as Glu-T currents on astrocytes before and during changing the perfusion rate.

Data were analyzed if the recorded neurons had resting membrane potentials negatively more than −60 mV, and the astrocytes had the resting membrane potential at −90 mV. The criteria for an acceptance of each experiment also included less than 5% changes in the resting membrane potential, spike magnitude, input resistance and series resistance throughout each of experiments. Input and series resistances were monitored by measuring cell responses to the hyperpolarization pulses at the same values as the depolarization that evoked spikes. The spikes, sEPSCs and Glu-T currents were presented as mean ± SE (Chen et al., 2010a; Ge et al., 2011; Yu et al., 2011). The statistical comparisons of such measured values under the conditions of control, ischemia and ischemia plus 3,5-DHPG application were done by *t*-test.

3. Results

3.1. Ischemia leads to an immediate impairment of cortical astrocytes

The astrocytes in cortical slices were recorded under voltage-clamp. Their Glu-T currents were induced by stimulating the presynaptic axons. We analyzed the dynamical changes of Glu-T currents before (control) and during reducing ACSF perfusion rate (ischemia).

Fig. 1 illustrates the dynamical changes of astrocytic Glu-T currents under the control and subsequent ischemia. The superimposed waveforms of Glu-T currents in Fig. 1A are an example under control (red trace) and after ischemia for 1 min (cyan), 3 min (blue) and 5 min (dark-blue). The averaged values of Glu-T currents at these astrocytes ($n = 13$) are 99.43 ± 2.76 pA under the control (red bar), as well as 60.9 ± 2.95, 58.68 ± 3.52 and 53.36 ± 3.8 pA after ischemia for 1 min (cyan), 3 min (blue) and 5 min (dark-blue), respectively (Fig. 1B; asterisks, $p < 0.01$). Therefore, the function of cortical astrocytes is impaired one minute after ischemia. As Glu-T function relies on ATP, our result indicates the high sensitivity of astrocytic Glu-T to ATP metabolism.

3.2. Ischemia impairs the functions of cortical GABAergic neurons

The responses of cortical GABAergic neurons to excitatory synaptic inputs were evaluated by recording their spontaneous excitatory postsynaptic currents (sEPSC) under whole-cell voltage-clamp (Wang, 2003; Wang and Zhang, 2004). sEPSC amplitudes were analyzed to present the neuronal responses to excitatory synaptic inputs.

Fig. 2 shows the effect of ischemia on sEPSCs at cortical GABAergic neurons. Compared to the control (red trace in Fig. 2A), sEPSC amplitude and frequency appear lower in ischemia (dark-blue). The averaged sEPSC amplitudes at these neurons ($n = 9$) are